INTERFERENCE OF MOONLIGHT WITH THE PHOTOPERIODIC MEASUREMENT OF TIME BY PLANTS, AND THEIR ADAPTIVE REACTION

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Abstract.—Threshold values of photoperiodic time-measurements correspond approximately to moonlight intensities. Experiments with *Glycine* and *Euglena* reveal that this is also the threshold value for synchronization of the circadian cycle. Saturation of this reaction is reached with 10 lx in 12:12 hr light-dark cycles. Thus, moonlight might disturb time measurement.

In *Glycine*, *Arachis*, and *Trifolium* the intensity of the light coming from the moon to the upper surface of the leaf is reduced by circadian leaf movement to values between 5 and 20 per cent (or even less than 5 per cent) of full-moon light intensity. Such a reduction eliminates the disturbing effects of moonlight. This finding indicates that leaf movements have an adaptive value of the kind that Darwin sought to identify. It also indicates that the behavior of the upper leaf epidermis as a "sense organ for light"[13] has an adaptive value.

In the short-day plants *Perilla ocymoides* and *Chenopodium amaranticolor*, a specific photoperiodic phenomenon was found that counteracts the disturbing effect of moonlight. Here light intensities similar to those of moonlight, introduced during the night, promote flowering instead of inhibiting it.

Threshold Intensities.—Usually the threshold value of light intensity in photoperiodic time-measurement corresponds approximately to the intensity of moonlight. Intensities as low as 0.1 lx may influence photoperiodism in plants and animals. The light reactions are sometimes saturated by intensities not higher than 5 lx and in other cases by intensities between 10 and 100 lx. Thus, with intensities beyond saturation, the photoperiodic reactions no longer depend on the light intensity.[1–4] Low threshold values of light intensity have an adaptive value, since normally the rate of change of light intensities is greatest in the morning and in the evening during that part of twilight characterized by very low light intensities. This means that possible disturbances through differences in cloudiness are at their lowest value during these periods of morning and evening twilight.

Threshold values for synchronization of circadian cycles by light-dark cycles have the same low values. This is again advantageous to the organism because if the phase-angle difference between these two cycles showed strong variations from day to day, the clock would run incorrectly. Since only a few experimental data on this matter are available, experiments with soybeans (*Glycine max*, var. *Gatersleben "H 7"*) were carried out. A weak synchronization of the circadian leaf movements with 12:12 hr light-dark cycles was still possible with intensities of 0.6–0.8 lx given during the light period. Saturation was reached with 10 lx when the free-running period was completely entrained to 24.0 hours (26.5 hr in continuous light), and the normal phase-angle difference between the light-dark cycle and the circadian cycle[5a] was retained. We have found the same values for the threshold and for saturation in the *Euglena* clock.[5b]

The highest photoperiodic responsiveness to light breaks is known to occur during the "subjective midnight-point" of the circadian cycle.[6] Response curves indicate that the greatest responsiveness to phase shifts of the circadian cycle also occurs in the vicinity of this point.

Thus, the threshold intensities are similar for the two light reactions involved in those photoperiodic timing processes that are performed with the help of the circadian clock. These low-threshold intensities are significant in adaptation. It must be noted, however, that this low-threshold value may permit disturbances of the timing process by moonlight. With the altitude of the moon at 60°, the intensity may reach 0.7 lx.[6] With the moon at still higher altitudes in tropical or subtropical regions, the maximum intensity may reach 1 lx. Thus, moonlight can influence photoperiodic reactions.[7] The strongest interference would be expected to occur in tropical regions at full moon at midnight, i.e., 18 hours after sunrise. In most species the "subjective midnight-point" of the circadian cycle, i.e., the point of greatest photoperiodic sensitivity to light breaks, is reached 16–18 hours after sunrise.[8] Since moonlight also reaches its highest possible intensity at this time, the organism may react to a night with a full moon as it would to a long day. In addition, phase shifts may result, so that the clock may run incorrectly.

Certain species of plants are apparently able to avoid such misinterpretations of the correct information.

Role of Leaf Movements.—Darwin[9] wrote that "when leaves or leaflets change their position greatly at night and by complicated movements, it can hardly be doubted that these must be in some manner beneficial to the plant .... Generally, the position which the leaves occupy at night indicates with sufficient clearness that the benefit thus derived is the protection of their upper surfaces from radiation into the open sky." Thus, Darwin assumed that leaf movement protects the plant against chilling. This assumption cannot be considered satisfactory since it is not applicable to plants living under tropical conditions, and leaf movements are most extreme in tropical plants. The present paper suggests that the adaptive value of leaf movement is not protection of the leaves against radiation from their surfaces *into* the sky, but rather protection of the leaves against radiation *from* the sky, i.e., from moonlight. The present experiments were made to test the validity of this suggestion.

The experimental plants were placed in growth chambers in such a way that the light source (bulb) was approximately vertically above the plants. The light intensity was reduced by layers of paper so as to result in an intensity of 1 lx at the vicinity of the leaves, thus simulating full moon conditions in tropical regions. The light intensity was at least three times higher than the intensity of moonlight measured with the same photocell in Tübingen. This refers also to the fraction of red light between 550 and 700 nm. The intensity of light reaching the upper surface of the leaves was measured with the help of photocells positioned in the plane of the leaves. In the case of very small leaves (*Trifolium*), additional paper models of the same transparency as the leaves were used. Although not very accurate, these measurements give some values from which to judge the real conditions. Table 1 shows some of the results, including the strongly reduced intensity of the light reaching the leaves when they are in their night position. This reduction must be less with lower altitudes of the moon. But with lower altitudes the interference with photoperiodic time measurement is less extreme, as mentioned above. Reduction of the light intensity reaching the leaves brings it below the known threshold values of photoperiodic reactions.

TABLE 1. *Light intensity in lx from an "artificial moon" reaching the upper surface of the leaves.*

Position of leaves	Glycine max	Arachis hypogaea	Trifolium repens
Day	0.8–1.0 (20)	0.6–1.0 (14)	0.7–1.0 (12)
Night	0.1–0.2 (20)	0.05 (14)	0.05 (12)

The intensity in a horizontal plane in the vicinity of the leaves is always 1 lx. The table compares intensities of light at the leaves in either day or night position of the leaves. The number of leaves or leaflets is given in parentheses.

This reduction is of course strongest with species that fold their leaves during the night. We have some experimental indications that, as in *Kalanchoe*,[11] and in other short-day plants also, the upper leaf surface shows a stronger responsiveness to light breaks than the lower one. If this is a general rule, the folding of the leaves must reduce the intensity of light reaching the photoperiodically most sensitive parts of the plant to values lower than 0.05 lx. Darwin[9] described nyctinastic movements of species that "hide" the upper surface of the leaves during the night. "Thus with *Cassia*, the leaflets which are horizontal during the day not only bend at night vertically downwards with the terminal pair directed considerably backwards, but they also rotate on their own axes, so that their lower surfaces are turned outwards." The present observations may indicate where one must search for the adaptive value of such complicated leaf movements.

Two more observations are of interest in this context: (1) according to experiments by Schwabe,[12] "The epiderm must play some direct or indirect role in the photoperiodic response mechanism in *Kalanchoe*." (2) Haberlandt[13] studied the specific anatomical and optical properties of the leaf epidermis, including leaves with "ocelli." He concluded that the upper epidermis functions as a sense organ for light. Only such a decisive role of the upper epidermis can explain the strange fact that photoperiodic action spectra correspond approximately to the absorption spectrum of phytochrome. This correspondence holds in spite of the very low concentration of phytochrome compared to the amount of chlorophyll present in the leaf. In most cases the upper epidermis does not contain chlorophyll, whereas, in many instances, the cells of the lower epidermis have chloroplasts. Thus, the very old botanical problem of a possible adaptive value of the lack of chlorophyll in the epidermis may finally be solved.

Role of a Specific Reaction in Plants to Moonlight.—While continuing our experiments on differences in responsiveness of the upper and lower surfaces of leaves to low light intensities,[11] we observed a photoperiodic phenomenon that may be

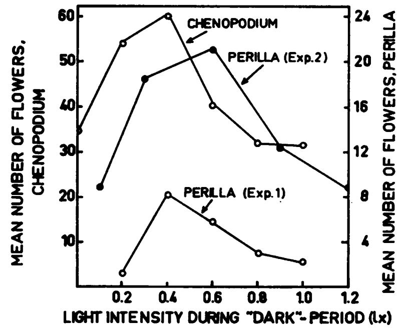

FIG. 1.—Influence of low light intensities on flowering in *Perilla ocymoides* and *Chenopodium amaranticolor*.

Abscissa: light intensity during the "dark-period" in lx.

Ordinates: median number of flowers per plant.

In the case of *Chenopodium*, the plants were kept under long-day conditions beginning with germination. To induce flowering, plants with four nearly expanded leaves were brought for 10 days into growth chambers (20°C). Light period: 10.5 hr, 2000 lx; dark period: 0–1.0 lx, white fluorescent tubes. Average values from 12 plants 5 weeks after beginning of induction.

In the case of *Perilla*, treatment also started with a long day. Inductive short days were offered when the plants had three pairs of leaves, the youngest being half expanded. Short-day conditions were as in the case of *Chenopodium*, but "dark-periods" 0.1–1.2 lx. Inductive conditions for 12 days (expt. 1) or 13 days (expt. 2). Median values from 12 plants (expt. 1) or 8 plants (expt. 2). Data represent number of flowers per plant 4 weeks after beginning of induction.

of interest in the present context. The short-day plants *Perilla ocymoides* and *Chenopodium amaranticolor* were raised in long days. The plants were then put into growth chambers under short-day conditions to induce flowering. During the short-day induction period, however, some plants were exposed during the long nights to low light intensities between 0.1 and 1.2 lx. The intensities actually reaching the leaves varied according to the variations in position of different leaves. Mutual shadowing in certain plants and artificial fixing of the leaves in certain positions with the help of wires added to these variations. If, for example, the value of 0.3 lx is given, the light intensity reaching the leaves varied between 0.1 and 0.6 lx. Figure 1 shows that low intensities do not inhibit flowering but promote it. The strongest promotion is found at light intensities corresponding to those of full moonlight. The present results with *Perilla* agree with earlier observations on flower formation of this species in long days with low light intensities.[14, 15] The observation that moonlight may promote flowering in the short-day plant *Amaranthus retroflexus*[8] should also be mentioned.

The promotion of flowering in short-day plants by exposing them to low intensity light at night prevents these plants from wrongly interpreting as a long day a moonlit night following a short day. These species do not interpret moonlight as "weak sunlight," but as "absolute darkness." This observation is of ecological interest, and involves physiological problems that deserve further study.

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